

## Microfilariae in the Free-Ranging Florida Panther (*Felis concolor coryi*)

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**ABSTRACT:** Blood samples from Florida panthers (*Felis concolor coryi*) collected from 1986 to 1993 during the months of December through May were screened for the presence of microfilariae (mff) by the Difil® filter test. Thirty-five of 47 (74.5%) panthers older than 2 yr of age were positive with microfilaremias ranging from 10 to 7,380 mff/ml of whole blood. No panthers that were 6 mo of age or less ( $n = 10$ ) were microfilariae-positive, and only 20% of the panthers in the 1-yr class ( $n = 5$ ) were positive. A representative number of microfilariae ( $n = 40$ ) from each of 7 freshly collected positive blood samples was measured and morphological characteristics were noted. The average length of microfilariae processed by the modified Knott's technique was 320  $\mu\text{m}$  (273–370  $\mu\text{m}$ ) with a width of 4–5  $\mu\text{m}$ . Of the 280 microfilariae measured, 202 (72.14%) had tapered heads and straight tails with an average length of 319  $\mu\text{m}$  (276–368  $\mu\text{m}$ ), 61 (21.79%) had blunt heads and straight tails and averaged 323  $\mu\text{m}$  (274–366  $\mu\text{m}$ ), 16 (5.71%) had tapered heads and button-hooked tails with an average length of 320  $\mu\text{m}$  (290–368  $\mu\text{m}$ ), and 1 (0.35%) had a blunt head and button-hooked tail and measured 320  $\mu\text{m}$ . The finding of no significant difference ( $P > 0.05$ ) between length measurements due to differences in head and tail shape leads us to believe that all microfilariae were of 1 species. Based on microfilarial length measurements, review of necropsy reports, and comparison with bobcat microfilariae, the most likely filarial species infecting the Florida panther is *Dirofilaria striata* (Molin, 1858).

**KEY WORDS:** Florida panther, *Felis concolor coryi*, *Dirofilaria striata*, microfilariae, morphology, prevalence, bobcat, *Lynx rufus*.

Filarial nematodes have been reported in a number of exotic felid species. *Dirofilaria repens* has been reported in the lion (*Panthera leo*) (Nelson et al., 1962). *Dirofilaria immitis* has been found in jaguars (*Felis onca*), tigers (*Felis sondiacus* and *Felis tigris*), wild cats (*Felis bangsi costariensis*), jagouarundi (*Felis yagouarundi*) (Otto, 1974), a bengal tiger (*Panthera tigris*) (Kennedy and Patton, 1981), and bobcats (*Lynx rufus*) from Florida (Levine, 1980; Forrester, 1992). Of 22 free-ranging mountain lions (*Felis concolor*) from California, 1 was seropositive for antibodies against the somatic antigen of *D. immitis* yet was seronegative for cuticular antigen and no circulating microfilariae (mff) were found in the whole blood (Paul-Murphy et al., 1994). *Dirofilaria striata* was first reported in Brazilian pumas (*Felis concolor* and *F. macroura*) (Railliet and Henry, 1911) and since has

been found in the ocelot (*Felis pardalis*), the margay (*Felis tigrina*) (Anderson and Diaz-Uribe, 1959), and the bobcat (Orihel and Ash, 1964; Miller and Harkema, 1968; Roelke, unpubl. 1985). Forrester et al. (1985) reported adult *D. striata* in mff+ adult Florida panthers.

The Florida panther is an endangered subspecies of cougar that inhabits pockets of habitat in the Big Cypress and Everglades ecosystems of southern Florida (U.S.A.). A population of less than 40 adult animals remains in the wild (Belden, 1986). Since veterinary involvement in the Florida Panther Recovery Program began in 1983, an intensive health evaluation and monitoring program has been in effect. The protocol includes the collection, screening, and storage of various biological samples each time an animal is immobilized. In the past, the presence of mff has been noted either by buffy coat analysis, by membrane filtration, or in reports from clinical laboratories. Adult *D. striata* were found in 4 out of the 7 panthers examined from 1973 to 1983, 3 of which were mff+ (Forrester et al.,

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1985). It was assumed that all microfilariae from these animals were representative of *D. striata* because no other adult filariids were detected.

Adult *D. striata* were found in 5 additional microfilaremic panthers at necropsy (Roelke, Nayer, and Vickery, unpub. data 1985). According to necropsy reports, 1–3 adult filariids were present singularly in the fascia between muscle bundles in the distal extremities. No reports of any filarial species other than *D. striata* were found in review of Florida panther necropsy reports ( $n = 41$ ) from 1980 to September 1994. This study was undertaken to determine the prevalence and microfilaremia of *Dirofilaria* sp. in the Florida panther population and to characterize and measure the mff in order to confirm the identity of the species of filariid present.

### Materials and Methods

From 1986 to 1993, a total of 83 blood samples representing 47 individual panthers and 3 bobcats was screened for the presence of mff. Samples were collected during the field capture season in the months of December through May from free-ranging panthers in the Big Cypress and Everglades ecosystems. All animals were anesthetized with ketamine hydrochloride (Ketastat<sup>®</sup>, Bristol Laboratories, Syracuse, New York, U.S.A.) or a combination of tiletamine/zolazepam (Telazol<sup>®</sup>, A. H. Robins Co., Richmond, Virginia, U.S.A.). Blood was drawn prior to administration of fluids, vaccinations, and prophylactic injections of Ivermectin (Ivomec<sup>®</sup>, Merck & Company, Incorporated, Rahway, New Jersey, U.S.A.) at a dose of 200 mcg/kg. Blood samples were taken directly from the saphenous vein into sterile 3-ml tubes containing ethylenediaminetetraacetic acid (EDTA) via a vacutainer and butterfly apparatus. The sample was kept cool in an insulated container for the 2–8-hr transition from field to lab.

One milliliter of EDTA preserved whole blood from each of the 17 samples collected in 1992 and 5 samples from 1993 was processed using the Difil<sup>®</sup> (EVSCO Pharmaceuticals, Buena, New Jersey, U.S.A.) procedure described by Howland and Todd (1977). In addition, 58 whole blood samples preserved in EDTA that had been collected from free-ranging panthers from 1986 to 1991 and stored in a 1:10 ratio of blood (1 ml) to 2% formalin (9 ml) at 4°C were screened for mff. From the 10-ml sample, 5 ml were passed through a Difil filter. Numbers of mff were determined for all positive samples (except FP#51 in which high numbers of mff/field made accurate counting impossible) by counting all individual mff on the filter at a viewing magnification of  $\times 200$  to obtain a mff/1 ml value. When no mff were found, the remaining 5 ml were tested in the same manner to confirm that the entire sample was indeed negative. In all cases, the Difil filter holder was washed and dried well between samples in order to avoid cross-contamination leading to false-positive results.

Of the 17 samples screened in 1992, 7 samples with

high numbers of mff were chosen for morphological study of individual microfilariae. All blood samples were kept in EDTA-coated blood tubes for the 2–8 hr between time of collection and time of microfilarial analysis. A 1-ml aliquot was processed using a modified Knott's procedure (Knott, 1939). Microfilariae were measured immediately after processing in order to minimize possible effects on length or width of microfilariae due to storage in 2% formalin. Measurements and morphological assessments were made with a calibrated ocular micrometer at a magnification of  $\times 400$ . Length, head shape, tail shape, and body shape were recorded for 40 mff/sample. Head shape was considered tapered if the width decreased upon successive measurements anteriorly (Fig. 1); if the sides remained parallel, the head was categorized as blunt (Fig. 2). Although many posterior ends were curved, only those that had a distinct hook at the tip were labeled as such (Fig. 1, 4).

Data were analyzed using SAS (SAS Institute Inc., 1989). Comparisons of total lengths of the microfilariae were made between panthers and bobcats and between morphological types using ANOVA with a split-plot design where felid type was the whole-plot factor and morphological type was the sub-plot factor. Three morphological types (tapered head and straight tail, blunt head and straight tail, and tapered head and button-hooked tail) were included in this analysis. Microfilariae with blunt head and button-hooked tails were not included due to low prevalence (1 found in panthers and 3 found in bobcats). Confidence limits were not calculated for percentage of prevalence of mff in panthers because the number examined represented nearly all of the extant population of panthers, thus rendering confidence limit calculations moot.

### Results and Discussion

Thirty-five (74.5%) of 47 adult ( $>2$  yr of age) Florida panthers sampled from 1986 to 1993 were mff+ at some point in their lives and all except for 2, which had low counts of 20 and 330 mff/ml, remained positive on subsequent tests. Because panthers were usually sampled at 2-yr intervals, the average age when animals become mff+ is not precisely known. None of the 5 panthers tested at 6 mo of age were mff+, 2 of 10 (20%) tested positive in the 1-yr-old class, and 15 of 23 (65%) of panthers were test positive at 2–4 yr of age. Of the 23 panthers that were 10 yr of age or older, 22 (96%) were mff+.

Counts ranged from 10 to 7,380 mff/ml of whole blood. Microfilarial counts showed fluctuations with no apparent trends when comparisons were made between animals or over periodic sampling of individual panthers. Administration of ivermectin at the time of capture seemed to have no effect on the long-term microfilarial intensities. Because the panther-capture interval was normally every 2 yr, it was

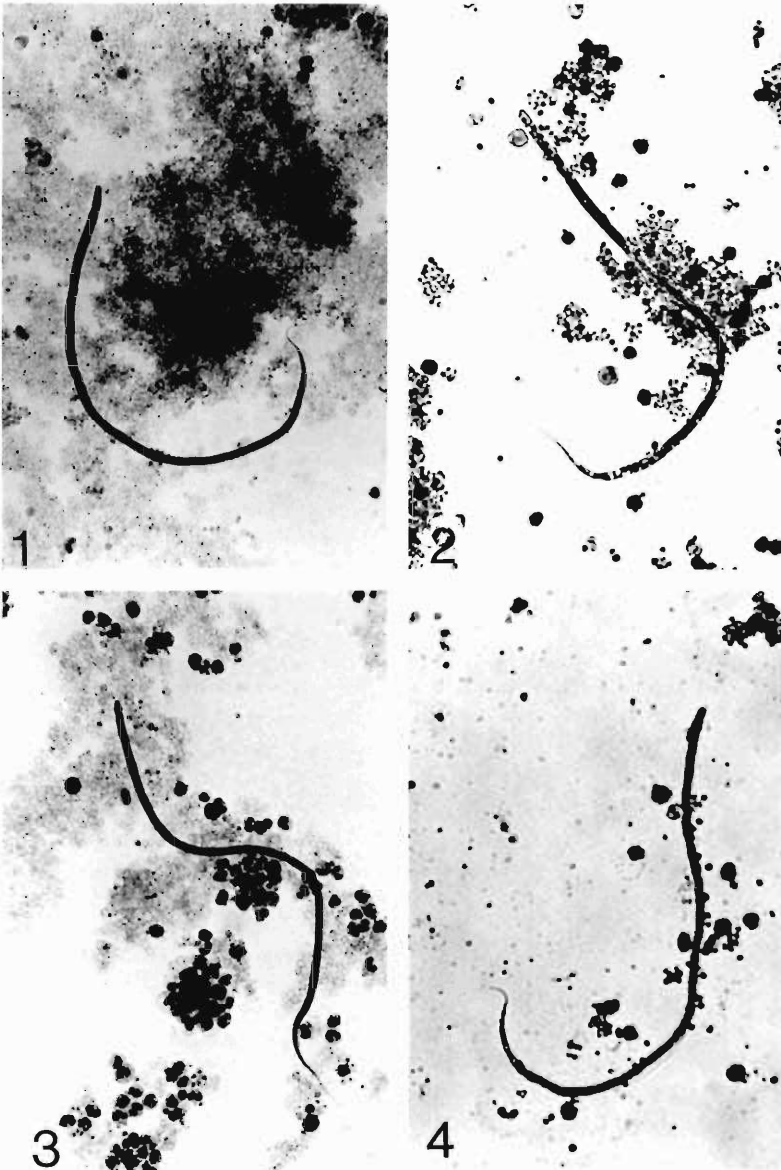


Figure 1. *Microfilaria* from panther blood with tapered head and hooked tail. Total length = 332  $\mu\text{m}$ .

Figure 2. *Microfilaria* from panther blood with blunt head and straight tail. Total length = 319  $\mu\text{m}$ .

Figure 3. *Microfilaria* from panther blood with tapered head and straight tail. Total length = 329  $\mu\text{m}$ .

Figure 4. *Microfilaria* from panther blood with blunt head and hooked tail. Total length = 323  $\mu\text{m}$ .

impossible to assess the short-term effect of this anthelmintic on microfilaremiias in the Florida panther.

Panther microfilariae were of 4 morphological types (Fig. 1–4): blunt head, straight tail (BS); blunt head, button-hooked tail (BH); tapered

head, straight tail (TS); and tapered head, button-hooked tail (TH). Microfilariae with tapered heads and straight tails were the most prevalent (72.1%), followed by those with blunt heads and straight tails (21.8%). The next most common were mff with tapered heads and button-hooked

tails (5.7%) and, lastly, those with blunt heads and button-hooked tails (0.4%). Body shapes were not used as criteria for distinguishing filarial types because shape appeared to be a highly variable parameter.

The average lengths of the mff from panthers measured in fresh blood samples ( $n = 280$ ) was  $320 \mu\text{m}$  ( $273\text{--}370 \mu\text{m}$ ). The widths ranged from 4 to  $5 \mu\text{m}$ . All of the mff measured remained in their original whole blood sample for a period of 2–8 hr before analysis. Courtney and Garber (1983) found, in measurement of *D. immitis* and *D. reconditum* mff collected from dogs and stored in EDTA for 8 hr, that there was not a significant change in microfilarial width or length. In an earlier study, Sawyer et al. (1963) determined that the width of *D. reconditum* stored in blood for 4–6 hours increased an average of  $0.5 \mu\text{m}$  above the average of those measured immediately. Both authors concluded that there was no change in morphological features during this time.

Because bobcats and panthers inhabit the same habitat and *D. striata* has been reported in the bobcat, a screening of 3 bobcat blood samples and comparison of mff between the 2 species was performed. Of the 3 bobcats examined, all had high microfilaremiias. The average length of bobcat mff measured ( $n = 120$ ) was  $333 \mu\text{m}$  ( $297\text{--}363 \mu\text{m}$ ) and the average width was  $5 \mu\text{m}$  ( $4\text{--}6 \mu\text{m}$ ). A majority of the mff (72.5%) had tapered heads and straight tails, 20% had blunt heads and straight tails, 5% had tapered heads and hooked tails, and 2.5% had blunt heads and button-hooked tails. This distribution of morphologic types is similar to that of the panther (see Table 1). Neither felid type, morphological type, nor the interaction between felid type and morphological type had a significant effect on microfilarial length ( $P = 0.3375$ ,  $0.3572$ , and  $0.4178$ , respectively).

Knott's procedure was the chosen method for studying morphological features because it caused less deformation and better delineation of the worms than did the Difil test. Jackson (1977) noted that the filtration step of the Difil test procedure seemed to cause shrinkage in length as compared to those measurements obtained from a Knott's preparation and that the filter membrane would often trap the mff midway through and obscure observation of the tail. The Difil method was used to obtain microfilarial counts because it was faster and was deemed

**Table 1. Distribution and average lengths of Florida panther and Bobcat microfilariae based on morphology.**

Head/tail	N	%	$\bar{x}$ ( $\mu\text{m}$ )	SD	Range ( $\mu\text{m}$ )
Tapered/straight					
Panther	202	72.14%	319	7.16	276–368
Bobcat	87	72.5%	333	6.16	297–363
Blunt/straight					
Panther	61	21.79%	323	8.25	274–366
Bobcat	24	20.0%	326	6.14	299–350
Tapered/button-hooked					
Panther	16	5.71%	320	8.52	290–368
Bobcat	6	5.0%	338	6.17	311–350
Blunt/button-hooked					
Panther	1	0.35%	320	0	320
Bobcat	3	2.5%	339	7.02	322–354

a more sensitive detection method by House and Glover (1974), who reported that the Difil test is 97.5% accurate whereas the Knott's procedure is only 89% accurate.

From review of necropsy data and the morphological data presented here, it can be inferred that *D. striata* is the only species of filariid present in the Florida panther. Comparison of the microfilarial length values with those published for various *Dirofilaria* spp. shows that they are most similar to *D. striata*, although references regarding measurements of this filariid in felids are few and reported microfilarial lengths vary significantly depending on processing methods. Anderson and Diaz (1959) isolated unsheathed mff from the uterus of adults with a length of  $235\text{--}270 \mu\text{m}$  and a width of  $5 \mu\text{m}$ . The measurements of formalin-fixed *D. striata* from bobcats reported by Orihel and Ash (1964) averaged  $348 \mu\text{m}$  ( $327\text{--}371 \mu\text{m}$ ) long by  $4\text{--}5 \mu\text{m}$  wide, and those measured from hematoxylin-stained thick blood films were  $230\text{--}240 \mu\text{m}$ . Redington et al. (1977) reported finding similar dimensions though no values were given. The finding of such a high prevalence of mff in adult Florida panthers warrants definitive microfilarial identification as well as continued screening of whole blood samples and investigation into the subtle health effects that such high numbers of circulating mff may pose.

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